REMARKS

With the entry of the present amendment, claims 1-16 and 20-32 are in this application. Claim 16 has been amended to incorporate the limitations of claim 17, and claims 17-19 are canceled without prejudice. Support for additional amendments to claim 16 is found, for example, at Examples 1, 2 and 4 of the as-filed application, which shows the steps for making the alphavirus replicon particle preparation, including the discarding of the culture medium. New claim 33 is supported by the specification at Example 1, page 29. None of the amendments made herein constitutes the addition of new matter.

The Requirement for Restriction

The Patent Office has stated that the process claims of the same scope as allowable product claims may be rejoined; thus the withdrawn claims are not canceled at this time.

The Telephone Interview

On July 20, 2009, there was a telephonic interview including the Examiner (Robert Kelly), Janice Kimpel of AlphaVax, inventor Kurt Kamrud of AlphaVax, Inc., inventor Sarah Caley (formerly Ian Caley) of AlphaVax, Inc., and the undersigned.

During this telephone interview, the particular advantages of the claimed preparations of alphavirus replicon particles encompassing expression libraries of tumor cells were discussed. The Patent Office was advised that the heparin binding nature of the particles was associated with the property of binding to the cells in which the particles were produced. These particles could be removed with a salt wash, where the wash solution is characterized by an ionic strength of 0.2 to 5 M. It was clarified by the inventors that the salt wash step in the method of making the preparation enabled the recovery of greater numbers of expressed sequences per unit size of experiment and therefore greater recovery of those sequences that are expressed at very low levels. This is due to the fact that prior

art methods of alphavirus replicon particles collected particles from the culture medium and at least 99% greater, and often 99.9% greater, of the particles produced had been discarded with the cells and cell debris. Thus, the present expression library preparations are inherently improved with respect to what range of sequences could have been represented in a preparation of particles containing a tumor cell expression library. In addition, the economics of producing such a library are dramatically reduced due to the hundred-fold or greater improvement in yield – less labor, less reagents and experimental procedures and products, and less costs associated with purification of particles from a relatively large volume of material and with handling extremely large volumes of cell culture, with its requirements for use of sterile technique and need for proper disposal. It was emphasized that the prior art methodology did not enable the present high quality products. The significantly improved yield was an unexpected observation by the inventors and their colleagues at AlphaVax, Inc., the assignee of the present application. The advantages of the claimed preparations and the methods for their production were discussed in the context of advantages over prior art preparations and methods.

Applicants appreciate the courtesy extended by Examiner Kelly in the interview and the discussion he provided.

The Nonstatutory Obviousness Rejections

Claims 16 and 32 have been rejected on the ground of nonstatutory double patenting as allegedly unpatentable over claims 1, 3-8, 10-15, 33-40, 44-49 and 51-77 of US Patent 6,521,235 in view of Slovin et al , Nestle et al. and Smooker et al. Applicants respectfully traverse this rejection.

Applicants respectfully submit that there is nothing in the claims of the cited patent which makes obvious the presently claimed invention, which relies on a particular method to produce an alphavirus replicon particle preparation

which encodes antigens corresponding to an expression library from an antigen source of interest, i.e. a tumor cell expression library. In part, it is the salt wash step which allows the production of such an alphavirus replicon particle preparation which corresponds to an expression library; the salt wash step for alphavirus replicon particles enables the release of a concentrated population of particles that is representative of the expression products of a tumor cell (claim 16) or a subtractive hybridization library of a tumor cell (new claim 33) and enables the efficient and reproducible recovery of rare members of such an expression library. The claims of the cited patent do not indicate that the population of particles corresponds to an expression library, such as that from a tumor cell, as set forth in the present application. Note the dependent claims that appear to indicate a single immunogen (or fragment).

Applicants also respectfully remind the Examiner that the present claims specify that the alphavirus replicon particles are heparin-binding. It is this property, not obvious over the cited patent claims or the additional nonpatent references, which results in particle binding to the host cells in which they are produced and subsequent release using a salt wash with a solution of 0.2 to 5 M ionic strength. The salt wash technique applied to the recovery of heparin-binding alphavirus replicon particles from producing cells results in a hundred- to a thousand-fold recovery of particles as compared to the prior art method of particle recovery from the culture medium. The process of the patent cited would have resulted in at least 99% and often 99.9%, or greater of the particles having been discarded, making the collected population of particles effectively enriched for the most common expression products. The prior art process would also have required the handling of 100-1000-fold more cultured mammalian cells and cell media to approach similar yields of alphavirus replicon particles. Handling such large volumes of cell cultures and media with such a low yield of commercially relevant doses is not considered economically feasible. The salt wash technique for preparing heparin-binding producing particles allows for the

production of surprising improved preparations, and in addition there is dramatic cost savings with respect to quantities of reagents, cDNA, starting tumor cell materials, replicon and helper nucleic acids, quantities of cultured cells and the costs associated with handling and disposing of large quantities of cell culture media in the prior art methods. This was discussed at length in the telephone interview with the Examiner on July 20, 2009.

In view of the foregoing, Applicants respectfully submit that the invention as claimed is surprisingly improved and not obvious over the cited art. The withdrawal of the rejection is respectfully requested.

Claims 16 and 32 have been newly rejected on the ground of nonstatutory double patenting as allegedly unpatentable over claims 32, 34-35, 37, 40, 42, 44-45, 47, 50-52, 54-55, 57, 60, 62, 64-65, 67, 72, 74-75, 77, 80, 82 and 84-90 of US Patent 6,531,135. Applicants respectfully traverse this rejection.

Applicants respectfully submit that there is nothing in the claims of the cited patent which make obvious the presently claimed invention, which relies on a particular method to produce an alphavirus replicon particle preparation which encodes a plurality of antigens corresponding to an expression library from an antigen source of interest. In part, it is the salt wash step which allows the production of such an alphavirus replicon particle preparation which corresponds to an expression library of the tumor cell and enables the efficient and reproducible recovery of the rare members of a tumor cell expression library. The claims of the cited patent do not indicate that the population of particles corresponds to an expression library from an antigen source of interest, such as a tumor cell, as set forth in the present application and claims. Note that the claims of the cited patent appear to indicate that there can be more than one antigen expressed from a single alphavirus nucleic acid, but not that the population encodes antigens corresponding to an expression library from an

antigen source of interest, e.g., a tumor cell, as claimed, as in the present application. In the present application, the population encompasses particles,which contain different replicon nucleic acids which collectively represent the sequences expressed by the antigen source, such as a tumor cell, while the cited patent contemplates a population of particles in which each particle contains the same replicon, whether it expresses one or multiple antigens.

In view of the foregoing, the withdrawal of the rejection is respectfully requested.

Claims 16 and 32 have been newly rejected on the ground of nonstatutory double patenting as allegedly unpatentable over claims 1-13, 16-17, 19, 23-35, 37-55, 57 and 61 of US Patent 6,156,558. Applicants respectfully traverse this rejection.

Applicants respectfully submit that there is nothing in the claims of the cited patent which make obvious the presently claimed invention, which relies on a particular method to produce an alphavirus replicon particle preparation which encodes a plurality of antigens corresponding to an expression library from an antigen source of interest. In part, it is the salt wash step which allows the production of such an alphavirus replicon particle preparation which corresponds to an expression library. The claims of the cited patent do not indicate that the population of particles corresponds to an expression library from an antigen source of interest, as set forth in the present application and claims. Note that the claims of the cited patent appear to indicate that there can be two antigens expressed from a single alphavirus nucleic acid, but not that the population encodes antigens corresponding to an expression library from an antigen source of interest, such as a tumor cell, as in the present application.

The importance of the salt wash technique and the surprisingly improved results with respect to economy and depth of the library have discussed above, and that discussion is incorporated herein.

The present claims provide a solution to the problem of immunization which is different from the approaches taught in the cited patent claims and the cited nonpatent references. In view of the foregoing, Applicants respectfully maintain that the present claims are not obvious over the claims of the cited patent, taken in conjunction with the nonpatent references. Accordingly, the withdrawal of the rejection is respectfully requested.

Claims 16 and 32 have been newly rejected on the ground of nonstatutory double patenting as allegedly unpatentable over claims 22-26, 28-29, 31-34 and 36-37 of US Patent 6,541,010. Applicants respectfully traverse this rejection.

Applicants respectfully submit that there is nothing in the claims of the cited patent which make obvious the presently claimed invention, which relies on a particular method to produce an alphavirus replicon particle preparation which encodes a plurality of antigens corresponding to an expression library from an antigen source of interest. In part, it is the salt wash step which allows the production of such an alphavirus replicon particle preparation which corresponds to an expression library which includes rare members of that library, a preparation of alphavirus replicon particles that is inherently different from the prior art preparations. The claimed salt wash method of preparation results in the improved depth of the library and was only made possible by the increase in particle yield of greater than two orders of magnitude and the concomitant, dramatic improvements in the economy of production of such an alphavirus replicon particle preparation. As already noted herein, the particles are heparin binding, a property associated with the binding of the overwhelming majority of the particles to the cells in which they are produced, allowing one to discard the

large volume of spent cell culture media (which is the **starting** material for collection of particles in the cited patent), and then collect the particles by releasing them from being bound to the cell and cell debris. The claims of the cited patent do not make obvious that the population of particles corresponds to a representative tumor cell expression library or a subtractive hybridization library from a tumor cell, as set forth in the present application and claims.

In view of the foregoing, Applicants respectfully maintain that the present claimed invention is not obvious over the cited claims with the nonpatent references. Accordingly, the withdrawal of the rejection is respectfully requested.

Claims 16 and 32 remain provisionally rejected on the ground of nonstatutory double patenting as allegedly unpatentable over claims 23-25 of copending US Application 11/132,711, and the Examiner has deferred this rejection until the present application or the cited application is allowed.

In view of the foregoing, the instant claims as amended are not properly rejected under Section 102(e). Thus, Applicants respectfully request the withdrawal of this rejection.

The Rejections under 35 U.S.C. 103

Claims 16 and 32 have been rejected under 35 U.S.C. 103 as allegedly unpatentable over US Patent 6,156,558 (Johnston), Nestle et al. (1998) and Smooker (2000). Applicants respectfully traverse this rejection.

The cited Johnston reference is said to teach the use of similar alphavirus particles in vaccines and to demonstrate that particles are sufficient to produce immune responses against foreign gene encoded proteins, but Johnston is acknowledged to lack the teaching of a plurality of antigens or the use of cancer antigens.

Nestle is said to teach a cocktail of peptides used to produce cancer immunity and Smooker is said to demonstrate that a library of epitopes may be administered to develop an immune response. The Patent Office has concluded that it would have been obvious to make a plurality of alphaviral replicons encoding the different peptides of Nestle and that the artisan would have been motivated to do so to produce an immune response to cancer, using the method of Smooker instead of actual delivery of the polypeptides. It is also alleged that there would have been a reasonable expectation of success as Smooker had demonstrated that a plurality of antigens could have been delivered and Nestle taught that the plurality of peptides produced immune response to cancer.

Applicants have previously amended the claims to incorporate the method steps from base claim 1 and to specify that the population of heparin binding alphavirus replicon particles encodes antigens corresponding to an expression library from a tumor cell. An important aspect of the method used to produce the population of the present invention as claimed is the salt wash step, which allows one to obtain dramatically larger alphavirus replicon particle yields (up to two to three orders of magnitude) than were possible in prior art methods; this, at least in part, allows the production of a representative expression library, rather than one containing only the most abundantly expressed proteins from an antigen source of interest. The claim has been further amended to clarify that the cell culture medium is discarded, and then the particles are released from the collected cells and cell debris. This leads to the production of a preparation of alphavirus replicon particles which is surprisingly improved over the prior art in the depth of the library and which was not previously possible with prior art methods. Prior to the development of the salt wash method for an alphavirus replicon particle resulted in more than 99% of the particles being discarded. The hundred to thousand fold (i.e. > 99.9%) or greater recovery allows a deeper sampling, i.e., an efficient and reproducible recovery of even the relatively rare

expression products in the alphavirus replicon particle preparation. The cited references make no teaching of the need for improved preparations or methods. Moreover, the present claimed preparations are improved dramatically with respect to the costs of production and processing. The cited art provides other solutions to the problem of immunogenic preparations – peptide libraries, other types of DNA and delivery methods or other strategies for the use of alphavirus vectors.

An important aspect of the method used to produce the population of the present invention as claimed is the salt wash step, which allows one to obtain dramatically larger alphavirus replicon particle yields than was possible in prior art methods; this, at least in part, allows the production of a representative expression library, rather than one containing only the most abundantly expressed proteins from an antigen source of interest. Specifically, in the '235 patent, yields of ARPs in BHK cells were reported to range from 3×10^5 to 1×10^8 per ml (Column 15, line 43). Applicants found that yields from the methods taught in the '235 patent in Vero cells were less than those obtained in BHK cells. In contrast, Applicants' method produced yields on the order of $10^{10} - 10^{11}$ (see Tables 1 and 2; paragraph [0056]), which are 2-3 orders of magnitude larger than the method practiced in the '235 patent. This is taught nowhere in the cited patent.

In addition, the cited patent appears to be limited to antigens related to Marburg virus, while the present application relates to a wide variety of antigen sources. The cited patent does not appear to teach or suggest a mixed population of alphavirus replicon particles, but rather single antigen-expressing populations or a population consisting of particles in which several antigens are expressed from a single nucleic acid. With respect to paragraph 5 in col. 7, it is not the same to say one or more particles derived from one or more replicon nucleic acids encoding one or more Marburg virion proteins. This is not what is

claimed in the present invention, and nowhere does the cited patent appear to teach the creation of an expression library in alphavirus replicon particles using the methods incorporated into the claims.

The Smooker reference teaches the creation of a secreted peptide expression library to be administered as a DNA preparation; at page 2535 it is stated that the majority of in-frame peptides were less than 20 amino acids and 13% were greater than 50 amino acids, with a range from 1-115. Thus, the library is one of partial proteins, and by virtue of the necessity for in frame fusions, only 1 in 6 clones represents a portion of a protein expressed in the antigen source (i.e. Plasmodium). This is a very different approach than is taken in the present Specification or is claimed in this application.

The cited Nestle reference relates to vaccination of melanoma patients with peptide or tumor lysate pulsed dendritic cells. This reference does not teach or suggest the use of any sort of **tumor cell expression library** for immunizing a patient.

Combining the cited references, in the absence of hindsight, could give a DNA vector for expressing melanoma antigens, an alphavirus system for expressing a plasmodium peptide library, a lysate of plasmodium or single preparations for expressing hemagglutinin, green fluorescent protein, or a Lassa fever N antigen or the proteins themselves. There is nothing that would point the way to the present claimed invention, especially in view of the relatively low recovery of the alphavirus replicon particles prior to present Applicants' discovery of the dramatic increase in yield with the use of a salt wash of collected cells for recovery of alphavirus particles which are characterized by heparin binding. It is noted that the improved recovery is greater than two orders of magnitude. This allows for the recovery of sequences that are expressed at very low levels in the antigen source without the expense of hundred to thousand-fold large production

processes, with all the associated costs for processing large volumes of culture and spent medium, labor and reagents.

In view of the foregoing, Applicants respectfully submit that the invention as claimed is not prima facie obvious over the cited art, and the withdrawal of the rejection is requested.

Claims 16 and 32 have been rejected under 35 U.S.C. 103 as allegedly unpatentable over US Patent 5,866,553 (Donnelly), US Patent 6,156,558 (Johnston) and Smooker (2000). Applicants respectfully traverse this rejection.

Donnelly is said to have taught immune responses to papilloma virus via DNA constructs encoding papilloma gene products. The Patent Office has concluded that because several antigens are taught which may be used in combination, immunization was against cancer. Johnston is said to teach the use of similar alphavirus replicon particles in vaccines and that the particles are sufficient to produce an immune response against foreign gene encoded proteins. Smooker is said to teach a library of Plasmodium epitopes expressed via a **plasmid** library. The Patent Office has concluded that it would have been obvious to modify the composition of Donnelly to contain different antigens of HPV in the alphaviruses of Johnston, that there was motivation to provide immunity to HPV and cancer and that the artisan would have had a reasonable expectation of success as Smooker had taught libraries of particles could elicit immunity.

Applicants have amended the claims to incorporate the method steps from base claim 1 and to specify that the population of alphavirus replicon particles encodes antigens corresponding to an expression library from a tumor cell. A critical aspect of the method used to produce the population of the present invention as claimed is the salt wash step, which allows one to obtain

dramatically larger (at least two orders of magnitude) alphavirus replicon particle yields than was possible in prior art methods; this, at least in part, allows the production of a representative expression library, rather than one containing only the most abundantly expressed proteins from an antigen source of interest. The prior art did not allow for the recovery of rarely expressed sequences, without the expense and labor associated with 110- to 1000-fold larger volumes processed. Moreover, the cited references appear to have followed other paths to their particular desired ends.

Donnelly appears to teach monovalent and multivalent vaccines for preventing PV infection. The monovalent vaccine may be made by formulating DNA encoding HPV16 L1 protein or L2 protein, or L1 + L2 proteins. Alternatively, a multivalent HPV vaccine may be formulated by mixing DNA encoding L1 or L2 or L1+L2 proteins from different HPV types". Thus, Donnelly at most teaches 2 selected antigens in a given construct and teaches making cocktails of different constructs to achieve "multivalent' vaccines. Smooker teaches the expression of peptide epitopes via a plasmid library. Both of these approaches are different from that of present Applicants, and thus can be considered to teach away from the alphavirus replicon particle expression library approach. Johnston teaches the alphavirus replicon particle approach, but does not teach the use of the method steps recited in the claims as amended to prepare a particle preparation. These steps (notably the salt wash step) allow the production of a significantly larger number of particles from a comparable starting cell culture and thus, the production of a representative expression library where the possible members are numerous. It is only by hindsight reconstruction that the Examiner could have selected various aspects from the cited references to arrive at the obviousness rejection. In addition, the particles of Smooker were biolistic particles with a DNA vector, not the alphavirus particles of the present invention, which are believed to allow for more efficient expression due to the ability of the

virus envelope proteins to facilitate entry into cells and the amplification of the replicon RNA once inside the cells.

Thus, the present claimed invention has several significantly and unpredictably improved aspects: the depth of the expression library with respect to relatively rare expressed sequences, an inherently improved property **only** of the present claimed compositions, dramatically lowered costs of preparing the population of particles in terms of quantities of reagents, labor and time, quantity of cell cultures required (and the requisite sterile handling and environmental conditioning required), volumes of material to be processed and safely discarded to achieve the same number of particles in a particular preparation.

In view of the foregoing, Applicants respectfully maintain that the present invention as claimed is not obvious over the cited art, and the withdrawal of the rejection is respectfully requested.

Conclusion

In view of the foregoing, it is submitted that this case is in condition for allowance, and passage to issuance is respectfully requested.

If there are any outstanding issues related to patentability, the courtesy of a telephone interview is requested, and the Examiner is invited to call to arrange a mutually convenient time.

This amendment is accompanied by a Petition for Extension of Time (three months) with authorization to charge the amount of $1110, as required under 37 C.F.R. 1.17(a). It is believed that this response does not necessitate the payment of any additional fees under 37 C.F.R. 1.16-1.17 or any additional extension of time. If the amount submitted is incorrect, however, please charge the necessary amount due under the foregoing Rules to Deposit Account No. 07-1969.

Respectfully submitted,

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